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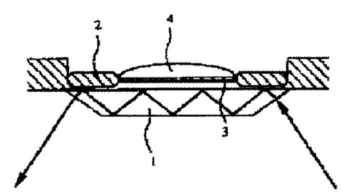
(54) **BIOCHEMICAL ANALYSIS DEVICE**

(57) Abstract:

PURPOSE: To measure enzyme activity via the infrared spectroscopic method by immobilizing a substrate to the surface of an attenuated total reflection prism directly or indirectly.

CONSTITUTION: A substrate is immobilized directly or indirectly to the surface of an attenuated total reflection prism 1, and a sample 4 is introduced to start reaction between the substrate and enzyme contained in the sample 4, thereby obtaining two infrared absorption spectra in different reaction time per sample. Then, the concentration change of the substrate or an enzyme reaction product in the sample 4 per unit time is calculated from the spectra, thereby measuring enzyme activity in the sample 4.



## DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application]This invention relates to the activity measurement method of the liquid sample which used infrared spectroscopy and attenuated total reflection spectroscopy, especially the enzyme contained into blood.
[0002]
[Description of the Prior Art]The activity measurement method of amylase is described in the 777th page among the enzyme activity measurement currently used with the conventional automatic analyser etc. from a clinical-examination-method manual, Izumi Kanai original paper (1983), and the 774th page. Here, add the starch which is a substrate into a blood serum, and it is made to react by a certain fixed time constant temperature, and the quantity of the reducing sugar generated at the substrate or enzyme-substrate reaction which remained is measured with a colorimetric assay method, and how to compute amylase activities is described.
[0003]It is related with the blood biochemistry analysis method using infrared spectroscopy and attenuated total reflection spectroscopy, and is applied. In the 95th page, it is discussed in spectroscopy (APPLIED SPECTROSCOPY) 1994 from the 48th volume and the 85th page. Here, the concentration of seven ingredients of glucose in human plasma, the quality of total protein, total cholesterol, triglyceride, urea, and uric acid is measured by infrared spectroscopy. It is related with measurement of the enzyme activity by infrared spectroscopy, and is bio-KIMIKA. Bio-FIJIKA The 242nd page is described from the 1159th volume and the 237th page in actor (BIOCHIMICA BIOPHYSICA ACTA) 1992. Here, the activity of alkaline phosphatase is relatively measured by the intensity measurement of the infrared-absorption peak of ****- nitrophenol phosphate which is a substrate.
[0004]
[Problem(s) to be Solved by the Invention]The reagent etc. which combined coloring matter, or the substrate and coloring matter for making a substrate and a substrate, or an enzymatic reaction product color are used for the enzyme activity measurement using the

conventional colorimetric assay method, its operation of mixing of a reagent, a reaction, etc. was complicated, and its cost of measurement was high. When measuring two or more enzymes, it was made to react individually using a specific reagent to each enzyme, and if it was ****, ** and analytical time were required. The biochemical analysis by the conventional infrared spectroscopy has an unnecessary reagent, and reaction time ******, Although measurement cost was cheap as compared with the conventional automatic analyser, it was impossible to have measured the activity of an enzyme for the measurement method etc. which measure one spectrum to the shortage of sensitivity and one sample originating in hard structure, and measure objective component concentration from a spectrum. Activity measurement was possible only for the enzyme in which the substrate which has a peak in a wavelength area without superposition at the infrared-absorption peak of an enzyme or an enzymatic reaction product in the measuring method of the enzyme activity by the conventional infrared spectroscopy exists.
[0005]By measuring the activity of an enzyme using infrared spectroscopy, the purpose of this invention is low cost and there is in providing the biochemical analyzer which measures enzyme activity for a short time.
[0006]
[Means for Solving the Problem]. [ whether a film which fixed a substrate is installed in attenuated-total-reflection prism in order to solve an aforementioned problem, and ] Or fix a substrate directly on the attenuated-total-reflection prism surface, and an enzyme and a substrate in a sample are made to react in an attenuated-total-reflection prism cell held at constant temperature, Two or more infrared absorption spectra in which reaction time differs about one sample are obtained, and enzyme activity in a sample is measured by asking for a substrate per unit time, or a concentration change of an enzymatic reaction product from an infrared absorption spectrum and a reaction-time difference.
[0007]
[Function]The above-mentioned means acts as follows. The enzyme substrate installed in attenuated-total-reflection prism directly or indirectly starts a reaction with the enzyme contained in a sample by pouring of the sample to an attenuated-total-reflection prism cell. The sample in an attenuated-total-reflection prism cell can detect the concentration change of the substrate by an enzyme reaction, or output with sufficient sensitivity in a short time by being maintained at the optimal temperature of an enzyme, producing a prompt enzyme-substrate reaction, and disassembling a lot of substrates for a short time with a thermostat. Since all specific substrates can be made to react to each enzyme by measurement once when making two or more enzymes into a measuring object, as compared with the conventional enzyme measuring method, analytical time is shortened substantially. By using infrared spectroscopy for the density measurement of a substrate or an enzymatic reaction product, enzyme activity can be measured by low cost, without using reagents, such as coloring matter currently used with the conventional biochemical analysis plan etc.
[0008]

[Example]Hereafter, this invention is explained in detail based on an example. Drawing 1 is a sectional view of the attenuated-total-reflection prism cell of the biochemical analyzer which is the first example of this invention. The attenuated-total-reflection prism 1 is formed from zinc selenide, germanium, silicon, or sapphire. The film 3 held at the frame 2 is installed via an about 1-mm gap on the attenuated-total-reflection prism 1. The films 3 are textiles films, such as cellulose, glass, or a synthetic resin.
It is a biochemical analyzer of this example, and when making amylase into a measuring object, on the film 3, the starch which is a substrate of amylase is fixed, for example. Immobilization of a substrate is performed by drying a solvent and making only a substrate hold on a film, after a film is immersed into a starch solution.
[0009]The sample 4 is poured in on the film 3 and penetrates the film 3. In that case, the starch which is the substrate fixed by the film 3 is eluted in a sample, and reacts to amylase. The sample containing the substrate eluted in the sample from the reducing sugar generated on the occasion of this enzyme reaction and the film 3 reaches on attenuated-total-reflection prism.
[0010]Drawing 2 is a block diagram of the biochemical analyzer which is the first example of this invention. This device can roughly be divided into the computer 11 which directs the operation of the test section 9 and the test section 9 which consists of the penetrant remover bottle 5, the spectroscope part 6, the liquid-sending mechanism 7, and the waste fluid bottle 8 via the control section 10. The spectroscope part 6 is composition which measures a spectrum by Fourier transform spectroscopy.
[0011]An operating personnel installs hand control or mechanically the film 3 which fixed the specific substrate in the enzyme of the measuring object on the attenuated-total-reflection prism 1, and introduces a sample by pipette or a syringe from the sample feed port 12 on the film 3. The introduced sample reaches on the attenuated-total-reflection prism 1, penetrating the film 3. An operating personnel directs a measurement start to the computer 11 through the final controlling elements 13, such as a keyboard and a mouse, further. The computer 11 to which the measurement start was directed sets fixed time, and measures two interferograms per one sample. Attenuated-total-reflection prism is installed into the thermostat 14.
While measuring two interferograms, sample temperature is held at 37 **.

[0012]An interferogram is changed into a digital signal by A/D converter 15, respectively, and is changed into a spectrum by the Fourier transform by the operation part of the computer 11. A spectrum is stored in the main memory of the computer 11 with measuring times. Two spectra stored in main memory are inputted into the measuring formula memorized by the external memory 16, and enzyme activity is computed. The operating personnel can read the value of the enzyme activity outputted to the indicators 17, such as CRT or a printer.
[0013]The film 3 is removed from on the attenuated-total-reflection prism 1 hand control or

mechanically after measuring finish. The penetrant remover in the penetrant remover bottle 5 is sent on prism by the liquid-sending mechanism 7 after that, and washing of the attenuated-total-reflection prism 1 surface is performed. The penetrant remover on prism is sent by the waste fluid bottle 8 with the liquid-sending mechanism 7 after the end of washing.

[0014]Drawing 3 is a sectional view of the attenuated-total-reflection prism cell of the biochemical analyzer of the second example of this invention. The composition of those other than the cell of this example is the same as that of drawing 2 of the first example. On the attenuated-total-reflection prism 1, an enzyme substrate solution is directly dried with **, and the film 18 is formed. A sample is directly introduced on the film 18 and the reaction of the enzyme in a sample and the substrate of the film 18 begins.

[0015]Drawing 4 is a sectional view of the attenuated-total-reflection prism cell of the biochemical analyzer of the third example of this invention. The composition of those other than the cell of this example is the same as that of drawing 2 of the first example almost.

[0016]The film 19 installed via an about 1-mm gap on the attenuated-total-reflection prism 1 is a metallic mesh like copper with sufficient for example, thermal conductivity.

The enzyme substrate is fixed by the surface by desiccation with **.

On the film 19, the cooling plate 20 and Peltier device 21 are installed via the gap. The sample 4 is introduced on the film 19 with a syringe or a pipette from the sample induction 22. Since the film 19 is a mesh with the aperture of 2 micrometers, a sample is made to penetrate on the prism 1, but the substrate fixed by the film 19 surface at that time melts into a sample, and the reaction of the enzyme and substrate which are contained in a sample is started. The temperature control element 23 is installed directly or indirectly in the prism 1 lower part by the prism 1 lower part.

The temperature of the prism lower part is kept constant.

The whole cell is covered with the thermal insulation 24, and the window material 25 with an ON emitting part of light transparent to an infrared region is installed. An infrared absorption spectrum is measured after the sample has been frozen by Peltier device 21.

[0017]In this attenuated-total-reflection prism cell, the measuring object component in a sample is condensed by freezing at the prism side, and the high sensitivity infrared absorption spectrum which is not influenced by the moisture in a sample can be obtained.

[0018]Drawing 5 is a figure showing the temperature change of the sample in enzyme activity measurement of one sample of the sample cell shown in drawing 4. Immediately after injecting a sample into a cell first, when cooling of a sample is performed and a sample reaches a predetermined temperature by a cooling element, a sample solidifies and the first spectrum is measured. After the first spectrum measurement, by sending reverse polar current through a Peltier device, sample temperature is raised, and it holds uniformly and is neglected for 5 minutes at 37 **. The reaction of the enzyme and substrate which are contained in a sample in the meantime advances. Polarity of the current which flows into a Peltier device again after neglect is made reverse, a sample is cooled, and the second

spectrum is measured.

[0019]Drawing 6 is a block diagram of the optical system of the spectroscope part of the first of this invention thru/or the third example. The spectroscope part 6 mainly comprises the light source 26, the interferometer 27, the attenuated-total-reflection prism 1, and the detector 28. The infrared light 29 emitted from the light source 26 enters into the attenuated-total-reflection prism 1 above a critical angle, after receiving abnormal conditions with the interferometer 27. Repeating total internal reflection within prism, the infrared light 29 which entered has the light of a specified wavelength absorbed by the sample 4 introduced on the prism 1, and is emitted to the detector 35. The light detected with the detector 28 is outputted to A/D converter 15 as the interferogram 30.

[0020]Drawing 7 is a flow chart of the proofreading method for measuring enzyme activity from two infrared absorption spectra in which reaction time with the substrate obtained about one sample differs. The reference solution group 33 which becomes the n measuring object material 31 from n reference solutions which added and created the specific substrate 32 to the enzyme of the measuring object is constituted. The infrared absorption spectrum 34 of each reference solution is measured with a sample cell and a spectroscope of the same type as this invention, and the substrate concentration 35 in another side each reference solution is correctly measured with a colorimetric assay method etc.

[0021]Substrate concentration in each reference solution which measured the absorbance of the wave number field which has a specific peak in a substrate with the infrared absorption spectrum of each reference solution with the explaining variable 36 and the colorimetric assay method is made into the purpose variable 37, multiple linear regression analysis or principal component analysis -- or partial It proofreads by the measuring method using multivariate analyses, such as a leased square (Partial Least Squares), (38), and the measuring formula 39 is computed.

[0022]The absorbance of the wave number field used for the above-mentioned proofreading of the second infrared absorption spectrum 42 measured m minutes after making it eluted with the first infrared absorption spectrum 41 measured immediately after having injected the sample into the sample cell and making the substrate 40 eluted in the sample 4 is substituted for the measuring formula 39, The substrate concentration 43 and 44 in each measuring time is computed. The enzyme activity 45 is computed from the difference of the substrate concentration of two more pieces, and the difference of the measuring time of a spectrum. The activity measurement method which measures enzyme activity is also possible by adding an enzymatic reaction product instead of a substrate by 32, constituting the reference solution group for measuring an enzymatic reaction product, and measuring the concentration of an enzymatic reaction product from the spectra 41 and 42.

[0023]Drawing 8 is a flow of the proofreading method for carrying out coincidence measurement of the activity of two or more enzymes in 1 sample. When conducting simultaneous analysis of two or more enzyme activity, all various kinds of substrates to

various kinds of enzymes of a measuring object are fixed on the film 3 of Example 1. Or one substrate is fixed on the film of one sheet, the film corresponding to various kinds of enzymes of a measuring object is made stratified in piles, and it installs on attenuated-total-reflection prism. Or on the attenuated-total-reflection prism surface, all various kinds of substrates to various kinds of enzymes for direct measurement are fixed.

[0024]When measuring the activity of the enzyme of q kind of measuring object, what mixed the substrate 46 (the substrate 1, substrate 2 --, the substrate q) of specific q kind with each enzyme is added to the n measuring object material 31, respectively, and it is considered as the n reference solutions 33. The infrared absorption spectrum 34 of each reference solution is measured with a sample cell and a spectroscope of the same type as this invention. On the other hand, the substrate 1 in each reference solution - the concentration of q are correctly measured with a colorimetric assay method etc. (47).

[0025]In the infrared absorption spectrum of each reference solution, the absorbance of a wave number field with a specific peak to the substrate 1 - q The explaining variable 48 (X1, X2 --, Xq), 1 in each reference solution measured with the colorimetric assay method - the substrate concentration of q as the purpose variable 49 (y1, y2 --, yq), It proofreads by the measuring method using multivariate analyses, such as multiple linear regression analysis, principal component analysis, or Partial Least Squares, (38), and 1 - the measuring formula 50 (f1(x), f2(x)--, fq(x)) about the substrate of q are computed.

[0026]The absorbance of the wave number field used for the above-mentioned proofreading of the second infrared absorption spectrum 42 measured m minutes after making it eluted with the first infrared absorption spectrum 41 measured immediately after making the substrate 50 of 1 which exists on the sample 4 and attenuated-total-reflection prism - q eluted is substituted for the measuring formula 50, 1 - the substrate concentration 52 and 53 of q in each measuring time are computed. The enzyme activity 54 is computed from the difference of the concentration furthermore computed two pieces to each substrate, and the difference of the measuring time of a spectrum.

[0027]Drawing 9 shows the result of having measured amylase in a blood serum using the third example of this invention, and the proofreading method of drawing 7. Soluble starch was fixed on the film as a substrate, and it installed on attenuated-total-reflection prism. The vertical axis was set as what computed the activity of amylase from the variation per unit of the starch concentration in a sample by infrared spectroscopy, and the horizontal axis was set as the value which measured the same sample with the Hitachi 7250 type automatic analyzer. The difference of the measuring time of two infrared absorption spectra was made into 5 minutes. The correlation coefficient of the 2 methods was as good as 0.99, and it was able to be measured by low cost in a short time, without the enzyme activity in a blood serum using reagents, such as coloring matter, by this invention.

[0028]

[Effect of the Invention]According to this invention, as compared with the enzyme activity measurement using the conventional colorimetric assay method, enzyme activity can be

measured by the short time and low cost.

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[Translation done.]

## CLAIMS

[Claim(s)]
[Claim 1]A biochemical analyzer having a film which fixed a substrate via direct or a gap on attenuated-total-reflection prism of the above-mentioned cell in infrared spectrometer containing a light source, an attenuated-total-reflection prism cell, a detector, and a computer for data processing.
[Claim 2]A biochemical analyzer making a substrate fix or stick to the attenuated-total-reflection prism surface of the above-mentioned cell in infrared spectrometer containing a light source, an attenuated-total-reflection prism cell, a detector, and a computer for data processing.
[Claim 3]Said film according to claim 1 is a textiles film formed from cellulose, glass, a synthetic resin, etc., or is 1.5 micrometers. A biochemical analyzer which is a mesh which has the above aperture.
[Claim 4]A biochemical analyzer provided with a mechanism for a cell of said attenuated-total-reflection prism according to claim 1 or 2 to keep temperature in a sample constant.
[Claim 5]A biochemical analyzer provided with a mechanism which a cell of said attenuated-total-reflection prism according to claim 1 or 2 cools sample temperature in a cell at at least 0 ** or less, and is measured.
[Claim 6]A biochemical analyzer with which, as for said attenuated-total-reflection prism cell according to claim 1 or 2, construction material of prism consists of zinc selenide, germanium, silicon, and sapphire.
[Claim 7]Two or more infrared absorption spectra in which reaction time of an enzyme and a substrate differs are obtained from one sample, The biochemical analyzer according to claim 1 or 2 which measures the activity of an enzyme contained in a sample by asking for a substrate for every reaction time or a concentration change of a resultant included in a sample from each infrared absorption spectrum.
[Claim 8]The biochemical analyzer according to claim 1 which made a measuring object two or more kinds of enzymes, and fixed all substrates to each enzyme on a film of one sheet.

[Claim 9]The biochemical analyzer according to claim 1 which made a measuring object two or more kinds of enzymes, fixed a substrate to each enzyme on a film of one sheet respectively, combined various kinds of films corresponding to two or more measuring object enzymes, and was installed on attenuated-total-reflection prism in piles in layers.
[Claim 10]The biochemical analyzer according to claim 2 which made a measuring object two or more kinds of enzymes, and fixed all substrates to each enzyme on the attenuated-total-reflection prism surface.

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[Translation done.]